Note

The Mre11p/Rad50p/Xrs2p Complex and the Tel1p Function in a Single Pathway for Telomere Maintenance in Yeast

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ABSTRACT

The Mre11p/Rad50p/Xrs2p complex is involved in the repair of double-strand DNA breaks, nonhomologous end joining, and telomere length regulation. *TEL1* is primarily involved in telomere length regulation. By an epistasis analysis, we conclude that Tel1p and the Mre11p/Rad50p/Xrs2p complex function in a single pathway of telomere length regulation.

IN *Saccharomyces cerevisiae*, Mre11p, Rad50p, and Xrs2p form a complex (Johzuka and Ogawa 1995; Usui *et al.* 1998), which we term the "MRX complex," required for several types of DNA repair and recombination (Kannar and Hoeijmakers 1997; Haber 1998). Null mutations in *MRE11*, *RAD50*, or *XRS2* lead to: (1) sensitivity to DNA-damaging agents, reflecting failure to repair double-stranded DNA breaks (DSBs) by homologous or nonhomologous recombination; (2) slow growth; (3) short telomeres; (4) defective meiotic DSB formation; and (5) elevated levels of spontaneous mitotic recombination (Haber 1998).

Although the exact biochemical role of the MRX complex is not clear, human Mre11p has nuclease activity that is increased by the addition of hRad50p (Paull and Gellert 1998); yRad50p exhibits ATP-dependent binding to DNA (Raymond and Kleckner 1993). Addition of hNbs1p (the probable human functional equivalent of Xrs2p) to the hMre11p/hRad50p complex results in several activities (unwinding of the DNA duplex and cleavage of fully paired hairpins) not observed in the absence of hNbs1p (Paull and Gellert 1999).

Mutations in the *TEL1* gene shorten telomeres, but do not result in a senescent phenotype (Lustig and Petes 1986). The closest structural homologue of *TEL1* is the human ATM gene, which is mutated in patients with the disease ataxia telangiectasia (Greenwell *et al.* 1995; Morrow *et al.* 1995). One conserved region between Tel1p and ATM is a C-terminal domain required for ATM kinase activity (Khanna *et al.* 1998). Although there is no direct evidence that Tel1p is a kinase, mutations of this region result in short telomeres (Greenwell *et al.* 1995), and Tel1p-dependent phosphorylation of Rad53p, RPA, and Rad9p in response to DNA damage has been observed (Brush *et al.* 1996; Sanchez *et al.* 1996; Emili 1998). The closest homologue of Tel1p in the *S. cerevisiae* genome is Mec1p (Greenwell *et al.* 1995; Morrow *et al.* 1995). Strains with a *mec1* mutation are sensitive to DNA-damaging agents (Weinert 1998) and have slightly shortened telomeres (Ritchie *et al.* 1999).

One method of attempting to define the functions of the various genes affecting telomere length is epistasis analysis, comparison of the phenotype of doubly mutant strains to strains with the individual single mutations. By this type of analysis, Tel1p and Yku70p function in separate pathways (Porter *et al.* 1996), as do Tel1p and Mec1p (Ritchie *et al.* 1999).

To examine genetic interactions between *TEL1* and the genes encoding the MRX complex, we constructed diploids heterozygous for the *tel1* mutation and *rad50* (KRY274), *mre11* (KRY277), or *xrs2* (KRY282) (Table 1). These strains were sporulated and the resulting tetrads were dissected. Since mutations affecting telomere length often exhibit phenotypic lag (Lustig and Petes 1986), we examined telomere lengths after subculturing the haploid strains derived from the spores. As shown in Figure 1, a–c, single mutations in *TEL1*, *RAD50*, *MRE11*, or *XRS2* all shorten telomeres to the same extent, as expected from previous studies (Kironmai and Muniyappa 1997; Boulton and Jackson 1998). Strains with the *tel1 mre11*, *tel1 rad50*, or *tel1 xrs2* genotypes have telomeres of the same length as observed for strains with the single mutations. This result indicates that all four genes are involved in a single pathway of telomere length regulation.

Strains with *mre11*, *rad50*, or *xrs2* mutations have sub-

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TABLE 1

Strain names, strain constructions, and relevant genotypes

Strain name	Strain construction or reference	Relevant genotype[a]
W303a	Thomas and Rothstein (1989)	Wild-type, *a* mating type
W303α	Thomas and Rothstein (1989)	Wild-type, α mating type
W303	Cross of W303a and W303α	Wild-type diploid
W303aU	Porter *et al.* (1996)	*a yku70::URA3*
SPY40	Porter *et al.* (1996)	*a tel1::URA3*
Y604	Provided by Y. Sanchez and S. Elledge	*a mec1-21*
KRY77	Spore derivative of KRY254	*a rad50::hisG*
KRY78	Spore derivative of KRY254	α *rad50::hisG*
KRY88	W303α transformed with *mre11::kanMX* cassette[b]	α *mrell::kanMX*
KRY97	W303α transformed with *xrs2::kanMX* cassette[c]	α *xrs2::kanMX*
KRY254	Transformation of W303 with *Bgl*II- and *Eco*RI-digested pNKY83[d], followed by isolation of 5FOA^R derivative	*a*/α *rad50::hisG/RAD50*
KRY272	Cross of KRY78 and W303aU	*a*/α *yku70::URA3/YKU70 rad50::hisG/RAD50*
KRY274	Cross of KRY78 and SPY40	*a*/α *tel1::URA3/TEL1 rad50::hisG/RAD50*
KRY275	Cross of KRY78 and Y604	*a*/α *mec1-21/MEC1 rad50::hisG/RAD50*
KRY277	Cross of KRY88 and SPY40	*a*/α *mre11::kanMX/MRE11 tel1::URA3/TEL1*
KRY278	Cross of KRY88 and Y604	*a*/α *mre11::kanMX/MRE11 mec1-21/MEC1*
KRY282	Cross of KRY97 and SPY40	*a*/α *xrs2:kanMX/XRS2 tel1::URA3/TEL1*
KRY283	Cross of KRY97 and Y604	*a*/α *xrs2::kanMX/XRS2 mec1-21/MEC1*

[a] All strains in the study are isogenic (except for changes introduced by transformation) with W303a (*a leu2-3,112 his3-11,15 ura3-1 ade2-1 trp1-1 can1-100*) (Thomas and Rothstein 1989).

[b] The *MRE11* gene was disrupted with the *kanMX* cassette by using the PCR-based method described by Wach *et al.* (1994). The oligonucleotide sequences were 5′ GACAATTGACGCAAGTTGTACCTGCTCAGATCCGATAAAACTCGACTCGTACGCTGC AGGTCGAC and 5′ GGTTATAAATAGGATATAATATAATATAGGGATCAAGTACAAATCGATGAATTCGAGCTCG.

[c] The *XRS2* gene was disrupted with *kanMX* cassette as described for the *MRE11* gene, except that we used the following primers:
5′ GTAATAGATGAGCAACAATACTGAGAAGGTGATAACTATAAATTTCGTACGCTGCAGGTCGAC and
5′ GCAAAATATAATTTAATGAAATTGGAAATACTCGGAAAATTTATCAATCGATGAATTCGAGCTCG.

[d] Alani *et al.* (1989).

stantially reduced growth rates (Alani *et al.* 1990; Haber 1998), whereas *tel1* strains grow at wild-type rates (Lustig and Petes 1986). The growth rates of the *tel1 mre11*, *tel1 rad50*, or *tel1 xrs2* are approximately the same as those observed for the single mutant *mre11*, *rad50*, and *xrs2* strains (Figure 1d). In addition, plating efficiencies (relative to a normalized value of 100% for wild type) were similar for *rad50* (64% with 95% confidence limits ±5%) and *tel1 rad50* (67% ± 7%) strains; *tel1* strains had approximately the same plating efficiency as wild type (96% ± 5%).

We previously showed that *tel1 mec1* strains had a senescent phenotype and telomeres that were slightly shorter than those of the *tel1* single-mutant strains (Ritchie *et al.* 1999). We constructed diploids that were heterozygous for *mec1-21* and *rad50* (KRY275), *mre11* (KRY278), or *xrs2* (KRY283). We found that spores with the *mec1-21 rad50*, *mec1-21 mre11*, or *mec1-21 xrs2* genotypes had a senescence phenotype that was indistinguishable from the *tel1 mec1-21* spores analyzed previously (Figure 2). After repeated subculturings, fast-growing "survivors" appeared in the *mec1-21 rad50* cultures; survivors also occur in telomerase-defective (Lundblad and Blackburn 1993) or *tel1 mec1-21* (Ritchie *et al.* 1999) strains. We also examined telomere lengths in wild-type, *rad50*, *mec1-21*, and *mec1-21 rad50* derivatives of KRY275. After 25 cell generations, the telomeres in the *mec1-21 rad50* strains were shorter than those in the *rad50* strain (data not shown). We conclude that the MRX complex functions in a different pathway from Mec1p, consistent with our previous conclusion that Tel1p and Mec1p represent different pathways.

Since the Tel1p and MRX complex are in a single pathway, but Tel1p and Yku70p are in separate pathways (Porter *et al.* 1996), Rad50p and Yku70p should function in separate pathways. In previous epistasis studies of the relationship between *rad50* and *yku70*, different conclusions were reached by different groups (Boulton and Jackson 1998; Nugent *et al.* 1998). We reexamined this issue by analyzing telomere lengths in spore cultures derived from a diploid (KRY272) that was heterozygous for *rad50* and *yku70* mutations. Telomere lengths in the double-mutant strain were shorter than those in either single mutant (Figure 3). In addition, spores of the double-mutant genotype grew more slowly than cells of either single-mutant strain (data not shown). These results demonstrate that *RAD50* (like *TEL1*) functions in a different pathway of telomere length regulation than *YKU70*, supporting the previous conclusion of Nugent *et al.* (1998).

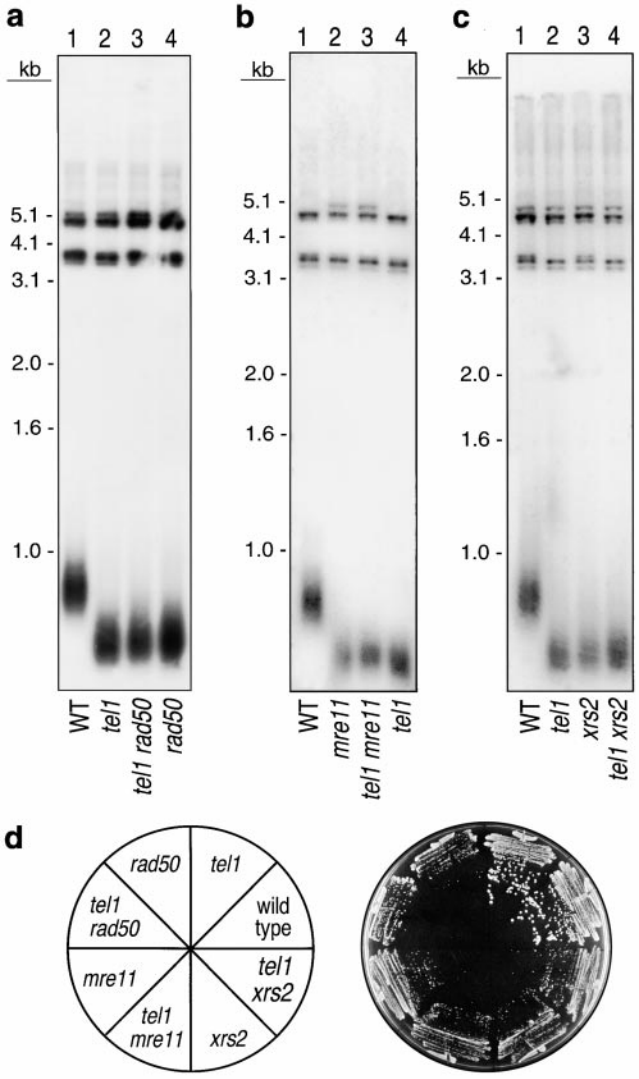

Figure 1.—Telomere lengths and growth rates in wild-type, *tel1*, *rad50*, *mre11*, *xrs2*, *tel1 rad50*, *tel1 mre11*, and *tel1 xrs2* strains. Diploids heterozygous for *tel1* and *rad50*, *mre11*, or *xrs2* were sporulated and dissected. Spore cultures were vegetatively subcloned four times (~80 cell divisions) on rich growth medium (YPD) at 30°. Genomic DNA was isolated from each strain and treated with *Pst*I. The resulting fragments were examined by Southern analysis, using a telomere-specific probe (Ritchie *et al.* 1999). The diffuse band below the 1-kb marker represents telomeric sequences, whereas the two bands at 3.5 and 4.8 kb represent tandem subtelomeric Y′ elements (Ritchie *et al.* 1999). Growth rates were qualitatively examined by streaking strains of various genotypes on rich growth medium (YPD), followed by growth at 30° for 2 days. All strains derived from spores were subcultured at least four times before analysis. Strain names and genotypes are as follows: (a) KRY274-3a (wild type), KRY274-3b (*tel1*), KRY274-3c (*tel1 rad50*), and KRY274-3d (*rad50*); (b) KRY277-2a (wild type), KRY277-2b (*mre11*), KRY277-2c (*tel1 mrell*), and KRY277-2d (*tel1*); (c) KRY282-3a (wild type), KRY282-3b (*tel1*), KRY282-3c (*xrs2*), and KRY282-3d (*tel1 xrs2*); and (d) W303a (wild type), SPY40 (*tel1*), KRY77 (*rad50*), KRY274-3c (*tel1 rad50*), KRY88 (*mre11*), KRY277-2c (*tel1 mre11*), KRY97 (*xrs2*), and KRY282-3d (*tel1 xrs2*).

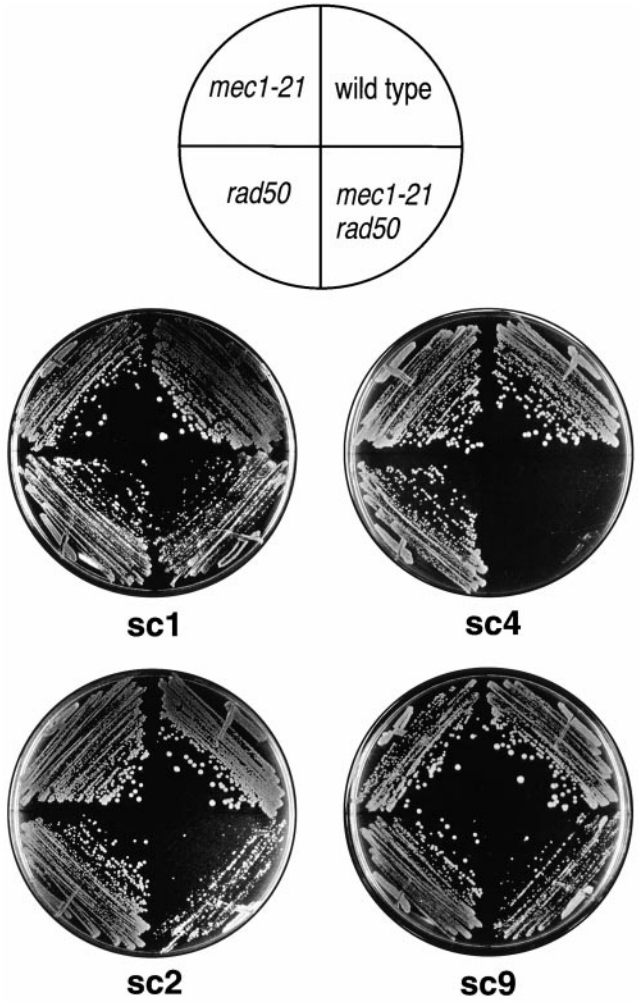

Figure 2.—Growth rates in wild-type, *mec1-21*, *rad50*, and *mec1-21 rad50* strains. The diploid strain KRY275 is heterozygous for *mec1-21* and *rad50*. This strain was sporulated and dissected. After spore colonies had formed, they were subcloned by repeated streaking (nine times) on YPD medium (Ritchie *et al.* 1999). The strain names and genotypes are as follows: KRY275-11a (wild type), KRY275-11b (*mec1-21*), KRY275-11c (*mec1-21 rad50*), and KRY275-11d (*rad50*). Plates from subclonings (SC) 1, 2, 4, and 9 were photographed. The *mec1-21 rad50* strain underwent senescence, with very poor growth by SC4. Survivors were generated by SC9. It should be noted that plates were usually incubated for 30° for 3 days before they were photographed. Under these conditions, the slower growth rate of *rad50* strains relative to wild-type strains is subtle.

What is the function of Tel1p and the MRX proteins in regulating telomere length? One obvious possibility is that these proteins directly activate telomerase catalytic activity. An argument against this possibility is that *tel1 tlc1* strains and *rad50 tlc1* strains have synthetic phenotypes different from those of the single mutants: *tel1 tlc1* strains senesce more *slowly* than *tlc1* strains (Ritchie *et al.* 1999), and *rad50 tlc1* strains accumulate postsenescence survivors more *slowly* than *tlc1* strains (Le *et al.* 1999). Thus, as described below, we prefer models in

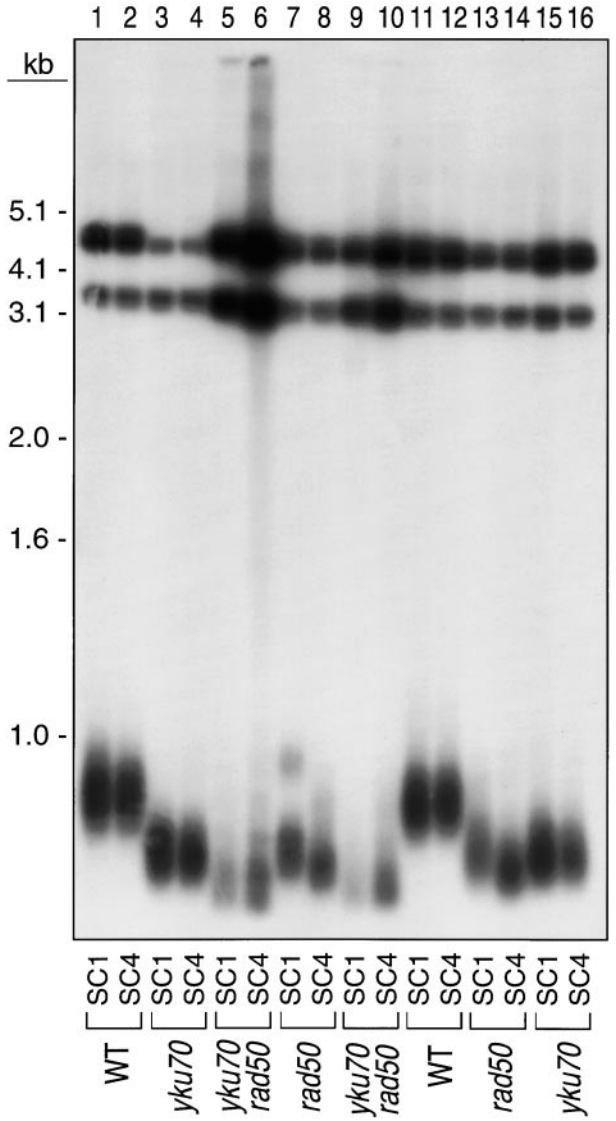

Figure 3.—Telomere lengths in wild-type, *rad50*, *yku70*, and *yku70 rad50* strains. A diploid strain (KRY272) heterozygous for the *rad50* and *yku70* mutations was sporulated and tetrads were dissected. Telomere lengths were analyzed in the spore products of two independent tetrads as described in the Figure 1 legend. Lanes 1 and 2, KRY272-8a; lanes 3 and 4, KRY272-8b; lanes 5 and 6, KRY272-8c; lanes 7 and 8, KRY272-8d; lanes 9 and 10, KRY272-15a; lanes 11 and 12, KRY272-15b; lanes 13 and 14, KRY272-15c; lanes 15 and 16, KRY272-15d.

which the Tel1p and the MRX proteins promote the activity of telomerase indirectly.

Since Tel1p has kinase motifs, one model is that Tel1p is required to phosphorylate one or more proteins of the MRX complex and that this phosphorylation is required for the role of the complex in telomere elongation. The role of the complex could be to "open" the telomere chromatin, allowing telomerase to interact with telomeric DNA. A related possibility is that the single-stranded poly $G_{1\text{-}3}T$ telomeric sequences could form a hairpin-like structure, and cleavage of this structure by a Tel1p-dependent phosphorylated MRX complex could increase accessibility of telomeric DNA to telomerase. Two further points should be mentioned. First, Tel1p and the MRX complex could affect accessibility of the telomere to cellular exonucleases as well as telomerase. Thus, in the absence of telomerase, *tel1* strains might have delayed senescence relative to strains with only a telomerase mutation (Ritchie *et al.* 1999). Second, since *tel1* mutants do not exhibit the growth deficiency or DNA repair defects shared by *mre11*, *rad50*, and *xrs2* strains, lack of phosphorylation by Tel1p does not affect all of the functions of the MRX complex.

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